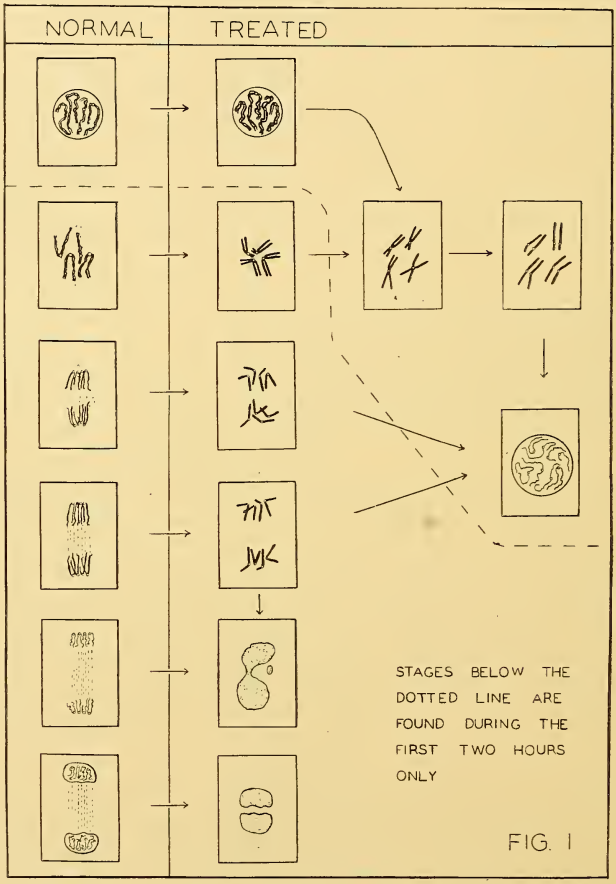
(3) There is no observable effect on interphase nuclei in colchicine treated material, but neither is there any real evidence about the time of initiation of the spindle, nor about the earliest point at which spindle suppression can occur. Cells treated during a division cycle do not reform a new spindle when colchicine is removed until they have passed through an interphase. Perhaps spindle suppression occurs at late interphase; this could explain why colchicine effects are still visible after 19 hours' recovery time.



Text-fig. 1.—Abnormalities resulting from the destruction of the spindle.

The time of such an action on interphase cells could not be accurately estimated without more information on the duration of stages in colchicine. As some diploid cells result after one hour's treatment the effect must be postulated in the later part of interphase, leaving cells in early interphase unaffected. This possibility would mean that the effect of colchicine is the same on all stages of division rather than one action on pre-metaphase stages and another on anaphase and telophase spindles. If spindle formation begins in interphase then the effect of colchicine is to destroy the spindle from the time of its initiation to telophase.

The nuclear material of cells in which the spindle is destroyed moved towards the centre of the cell. Apparently at anaphase and telophase in normal cells the spindle overcomes the forces which hold the nucleus at the centre of the cell, and keeps daughter nuclei apart until the new cell plate is formed. If the spindle is destroyed the original forces predominate once more and the nuclear material takes up a central position.

A diagrammatic representation of the effect of colchicine on cells in all stages of mitosis is shown in Figure 1.

Cell wall formation is prevented by colchicine, probably as a result of spindle suppression. No cell plates are formed if the spindle disappears at telophase. When the cell recovers, cell plates are formed after the spindle reappears, but not before, and a cell which becomes binucleate during treatment remains in that condition till it divides again during recovery. The observation that numerous cross walls form in recovered colchicine treated cells supports the view that cell plate formation is dependent on the spindle. The division figures of polyploid cells produced by treatment are frequently too wide to fit in the cell in the usual way, so they turn length-wise or from corner to corner in the cell. The cell plate in these cells forms across the spindle wherever it may be, not in the normal position across the cell.

In addition to altering the normal arrangement of chromosomes, colchicine affects their contraction since prophase and metaphase chromosomes are shorter and thicker than in normal cells. Two suggestions have been made to explain this aspect of the colchicine effect. Levan and Ostergren (1943) believe that colchicine, by destroying the spindle, prolongs metaphase and as a result spiralization continues for a longer time. This means thicker chromosomes are secondary effects of spindle destruction.

Ostergren (1944) rejected this explanation because he found thick chromosomes in prophase as well as metaphase, and also that chromosome contraction began at lower concentration than spindle suppression. He observed thickened chromosomes in some cells with normal or nearly normal spindles and concluded from this that colchicine must have a direct effect on the spiralization process. It is difficult to decide between these two views, especially since thickened chromosomes have been observed during this investigation in cells at anaphase after the spindle has been destroyed by colchicine. There seems to be some evidence to indicate a slowing down of some stages of the cell division process by colchicine. Metaphase is longer than normal when colchicine removes the spindle (Levan 1938; D'Amato 1948) and Barber and Callan (1943) found blocked metaphase chromosomes in swollen vacuolated newt cells treated with colchicine. Vacuolation normally occurs at the end of anaphase. Gaulden and Carlson have shown that colchicine not only prolongs metaphase but also prophase; so that extra spiralization at prophase could be due to a prolonged division with chromosome contraction proceeding at the normal rate. However, it is not clear how this could account for thicker chromosomes at anaphase.

All the observed irregularities indicate a disorganized cell division mechanism and can be explained by the absence of the spindle. The spindle appears to be responsible for metaphase plate formation, anaphase separation, holding the chromosomes apart at anaphase and telophase and for cell plate formation. Possibly it controls also the timing of the cell division process. In other words the spindle is responsible for the organization of the cell division and when it is removed the chromosomes continue their part in the division cycle but without the usual organization. It should be stressed that the formation of a tetraploid nucleus during colchicine treatment is not a reversal of any part of the division process, but a forward sequence of events following the normal path as closely as is possible without the spindle.

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EXPLANATION OF PLATE XI.

All photographs × ca. 350.

A, B.—Normal cells showing spindles. C, D.—Abnormal metaphase. Treated for 30 and 45 minutes with colchicine. E, F.—Colchicine treated telophase. E shows a partly developed cell wall but the spindles have been destroyed in both cells. G.—Two nuclei close together in the cell after colchicine treatment for 45 minutes. H.—Abnormal interphase nuclei—a dumb-bell-shaped nucleus and a binucleate cell. Thirty minutes' treatment. J, K.—Prophase in irregular nuclei after one hour in colchicine followed by 19 and 48 hours' recovery respectively in water. L.—Prophase showing early development of the spindle on either side of the nucleus. One hour in colchicine and 48 hours in water. M.—Spindle and cell plate in colchicine induced tetraploid telophase. One hour in colchicine, 48 hours in water.

A STUDY OF THE MICROFLORA OF WHEAT GRAINS IN NEW SOUTH WALES.

By Dorothy E. Shaw, Faculty of Agriculture, University of Sydney, and
P. G. Valder,* New South Wales Department of Agriculture.

[Read 26th November, 1952.]

Synopsis.

The microflora of surface sterilized wheat grains from 49 samples harvested in 1949 and 1950 was examined by means of plating tests. Germination tests in soil and brief non-quantitative examinations of the surface flora were also carried out.

Bacteria and *Penicillium* spp. constituted a large part of the surface flora, while *Alternaria* spp. were the fungi most commonly isolated from surface sterilized grains, exceeding the total of the other organisms isolated by almost three times. Of the other organisms isolated, only *Septoria nodorum, Helminthosporium sativum* and *H. tritici-repentis* are known to be pathogenic to wheat, and of these only *H. sativum*, which was isolated from approximately 0·5% of the grains, has been observed to cause a seed-borne disease. *Fusarium* spp. were isolated only very rarely and these isolates were not pathogenic to wheat under glasshouse conditions.

Studies were made of the distribution of mycelium within the grain, and of the internal floras of various atypical types of grain, viz., pinched, mustard, pink and black-pointed grains. A marked association was observed between a pink discoloration of the grain, and the presence of *H. tritici-repentis* within it. *Alternaria* spp. were isolated from a greater proportion of black-pointed grains than from apparently normal ones, but further work is needed to establish the cause of this condition as it occurs in New South Wales.

The factors affecting the population present and the economic importance of the microflora are discussed.

Introduction.

Numerous organisms have been recorded on and in wheat grains from all parts of the world. The literature relating to this subject is extensive and in this study is reviewed as briefly as possible, an attempt being made to discuss the various aspects in the light of results obtained from investigations on grain samples from New South Wales. The work is concerned mainly with organisms isolated from surface sterilized grains.

The aims of the study were

1. to examine the microfloras of samples from the 1949 and 1950 harvest, noting differences, if any, between samples from different parts of the State;
2. to observe any relationships which might exist between the organisms present, the appearance and germination of the samples, and any diseases which might develop on seedlings grown from these samples; and
3. to determine the position of fungi within the grain.

Organisms Previously Recorded.

One of the earliest observations was made by Bolley (1913) who stated that certain fungi could be obtained from the surface and interior of wheat grains from almost any wheat-growing region of the world.

The surface flora has usually been determined by shaking the grain with water and examining the washings under the microscope. Various workers have recorded numerous common moulds, mainly species of *Alternaria, Aspergillus, Cladosporium, Penicillium* and bacteria, together with pathogens such as *Tilletia caries, T. foetida, Ustilago tritici, Helminthosporium sativum, Septoria nodorum, S. tritici, Fusarium* spp. and rusts (Carter and Young, 1950; Christensen, 1951; Crosier, 1936; Greaney and Machacek, 1942, 1946; James et al., 1946; Pollack, 1945; Rice, 1939; Russell and Ledingham, 1941; Schnellhardt and Heald, 1936). The fungi are usually present as spores and generally are regarded

* Work undertaken while a student in the Faculty of Agriculture, University of Sydney.

merely as contaminants, but James et al. (1946) suggest that since bacteria occur in such large numbers they constitute an epiphytic flora.

The organisms recorded within wheat grains include species of *Alternaria*, *Aspergillus*, *Botrytis*, *Cephalosporium*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Epicoccum*, *Fusarium*, *Helminthosporium*, *Mucor*, *Nigrospora*, *Penicillium*, *Phoma*, *Podosporiella*, *Pullularia*, *Rhizoctonia*, *Rhizopus*, *Sclerotium*, *Septoria*, *Stemphylium*, *Torula*, *Trichoderma*, *Trichothecium*, *Verticillium*, together with various other moulds, yeasts, bacteria and actinomycetes (Atanasoff, 1920; Blair, 1937; Bolley, 1924; Brentzel, 1944; Brittlebank and Adam, 1924; Carne, 1927; Christensen, 1951; Curzi, 1926, 1929; Dastur, 1928, 1942; Davidson and Den Shen Tu, 1950; Drechsler, 1923; El-Helaly, 1947; Fomin and Nemlienko, 1940; Galloway, 1936; Greaney and Machacek, 1942, 1946; Hagborg, 1936; Henry, 1923; Laumont and Murat, 1934; Machacek, 1945; Machacek and Greaney, 1938; Machacek et al., 1951; McCulloch, 1920; Milner et al., 1947*a*, 1947*b*; Minz, 1943; O'Gara, 1915; Peyronel, 1926; Rosella, 1930; Russell and Ledingham, 1941; Weniger, 1935; Ziling, 1932).

Most of the studies on the internal flora have been made using discoloured samples, *H. sativum* and *Alternaria* spp. of the *A. tenuis* type being the organisms most commonly present. The general finding with ordinary, sound, market samples has been that *Alternaria* spp. are the most common internal fungi, in some cases occurring in almost every grain (Christensen, 1951; Davidson and Den Shen Tu, 1950; Greaney and Machacek, 1942, 1946; Hyde and Galleymore, 1951; Laumont and Murat, 1934; Machacek et al., 1951; Milner et al., 1947*a*, 1947*b*).

Greaney and Machacek (1946) made a thorough study of a large number of samples from Manitoba over the period 1937–42, and found that 70·2% of the grains were infected with *Alternaria* spp., 3·5% with *H. sativum*, 0·6% with *Fusarium* spp., 3·2% with other fungi, and that 23·9% of the kernels were fungus-free. Bacteria, yeasts and actinomycetes were also found.

Pleosphaeria semeniperda, *Penicillium glaucum*, *Penicillium* sp., *Cladosporium* sp., *H. sativum*, *Alternaria* spp., *Fusarium* spp., *Pseudomonas atrofaciens* and other bacteria have been recorded in Australia, but little detail is given (Adam, 1950; Anon., 1939; Brittlebank and Adam, 1924; Carne, 1927; Noble, 1924, 1933)

Materials and Methods.

Wheat samples were obtained from various parts of New South Wales. Samples 1–20 were from the 1949 harvest and samples 21–49 from the 1950 harvest. Samples 1–17 were from silos and were deliberately selected to contain a high proportion of discoloured grains, whereas Nos. 18, 19 and 20 were random samples. Numbers 21–49 were random samples collected from various sources.

The 1949 samples were subjected to an agar plate test six months after harvest. The 1950 samples were examined one month after harvest and, following Canadian proposals (Greaney and Machacek, 1946; Machacek and Wallace, 1942; Mead et al., 1950; Russell and Ledingham, 1941), were subjected to the following:

(*a*) a macroscopical examination to observe the degree of pinching and the proportion of discoloured, shot, sprung and mechanically injured grains;

(*b*) a qualitative examination of seed washings;

(*c*) an agar plate test;

(*d*) a non-sterile soil test to determine the germination and to observe any seed-borne diseases.

No attempt was made to detect loose smut infection.

The presence of the groove and the brush make the wheat grain admirably suited for carrying a large surface load of organisms, and for this reason a method of thorough surface sterilization is needed if the internal flora is to be examined by means of an agar plate test.

Various techniques have been employed (Machacek and Greaney, 1938; Mead, 1933; Simmonds, 1930*a*; Simmonds and Mead, 1935) and their relative merits are discussed

by Machacek and Greaney (1938) who devised what they describe as a simple, practical and efficient method, which they later (Greaney and Machacek, 1946) modified slightly. Except where otherwise indicated, this modified method, which is described below, has been adopted throughout this study.*

The grains were immersed in a solution of alcohol and mercuric chloride (1 part of 95% ethyl alcohol to 3 parts of 1:1000 mercuric chloride solution) for four minutes, washed in three changes of sterile water, and planted in freshly poured P.D.A. plates, 10 grains per dish. The dishes were held at room temperature for 8–10 days. At the end of this period the organisms growing out from each kernel were either identified at once or isolated for further study and identification.

The germination tests in non-sterile soil were carried out according to the method described by Machacek and Wallace (1942) and Mead et al. (1950). 100 grains from each sample were planted in pots in a glasshouse and left for 10 days, the temperature ranging from 20 to 30° C. during that period. The seedlings were then counted, taken from the soil, washed, and examined for lesions. The diseased tissue, where present, was then surface sterilized and planted in P.D.A. plates, to determine the organisms responsible.

General Results.

Only a brief qualitative examination was made of the surface flora. Microscopical examination of the washings revealed spores of *Alternaria*, *Cladosporium*, *Epicoccum*, *Stemphylium*, numerous unicellular spores, uredospores and hyphal fragments. Occasional spores of *Tilletia* were also found. When the washings were streaked on to P.D.A., the organisms which developed were mainly bacteria and species of *Penicillium*, and it is probable that these constitute a large part of the surface flora.

The results of the plating tests to determine the internal flora of samples 1–17 are shown in Table 1. Species of *Alternaria* were the organisms most commonly isolated, exceeding the total of the other organisms isolated by almost three times. *Helminthosporium tritici-repentis* was isolated from 8·5% of the grains, *Septoria nodorum* from 2% and *H. sativum* from only 0·4%, while *Fusarium* spp. were not isolated at all. There appears to be little relation for these samples between germination in agar, the proportion of grains from which organisms were isolated, and the number and nature of the organisms, except that the two lowest germination figures were obtained with samples 11 and 12, from which were isolated the most colonies of *Aspergillus* spp. This is in keeping with the work of various authors (Laumont and Murat, 1934; Thomas, 1937) who found that *Aspergillus* spp. were able to reduce germination. Samples from which *H. tritici-repentis* was isolated were all from the northern half of the State, and it is interesting to note the high figure for *S. nodorum* obtained with the sample from Ladysmith.

The results obtained with random samples from the 1949 harvest are shown in Table 2, and are much the same as those obtained with samples 1–17, except for the high figure for *Penicillium* spp. in sample 18.

In Table 3 are grouped the results obtained with samples from the 1950 harvest. The population of organisms isolated was similar to that isolated from samples of the previous harvest, *Alternaria* spp. again exceeding the total of other organisms isolated by almost three times. *H. tritici-repentis* was prominent as before, being second in frequency to the *Alternaria* spp., this time, however, sharing the position with *Epicoccum* sp. *S. nodorum* was isolated from 2·7% of the grains, *H. sativum* from 0·5%, and a *Fusarium* sp. from only 0·1%.

It is clear, therefore, that the microflora of wheat grains in New South Wales, as revealed in these samples, is, except for the presence of *H. tritici-repentis*, very similar to that found overseas.

Alternaria spp. occurred in every sample examined, the percentage of grains from which they were isolated in any sample ranging from 16 to 95. Nearly all the isolates

* For justification of its use see the appendix.

TABLE 1.

The Organisms Isolated from, and the Germination in Agar of Samples 1–17 from the 1949 Harvest.

Sample.	Source.	Germination in Agar.	Number of Grains Giving—								
			Alternaria spp.	*H. triticirepentis.*	*Aspergillus* spp.	*Septoria nodorum.*	*Penicillium* spp.	*Rhizopus nigricans.*	*H. sativum.*	Miscellaneous.*	No Organisms.
1	Westdale..	38	29	13	—	1	1	—	—	—	6
2	Emerald Hill	46	30	12	2	—	—	1	1	—	5
3	Breeza	37	29	8	3	3	2	—	1	—	5
4	Manilla	41	16	7	—	—	1	—	—	—	27
5	Delungra..	42	30	5	—	—	—	1	—	—	15
6	Warialda..	42	38	7	—	1	—	—	—	7	1
7	West Tamworth	40	24	14	—	—	—	2	—	1	10
8	Inverell	39	31	—	—	—	—	4	1	7	9
9	Quirindi	34	37	6	—	—	—	4	1	1	2
10	South Harefield	32	33	—	7	—	3	1	—	2	8
11	Brushwood	19	28	—	18	—	—	—	—	1	3
12	Corobimilla	16	20	—	16	—	1	—	—	1	12
13	Ladysmith	31	25	—	14	10	—	—	—	4	2
14	Arajoel	32	37	—	5	—	2	—	—	—	7
15	Illabo	29	48	—	—	—	1	—	—	1	—
16	Old Junee	38	39	—	—	—	—	—	1	2	10
17	Coolamon	32	36	—	—	1	3	1	—	5	10
	Average	34·6	31·2	4·2	3·8	1·0	0·8	0·8	0·2	1·9	7·7
	Average (%)	69·2	62·4	8·5	7·6	2·0	1·6	1·6	0·4	3·8	15·4

50 grains plated from each sample.

* Includes *Epicoccum* sp., *Cladosporium* sp., *Pullularia pullulans*, *Stemphylium* sp., *Botrytis* sp., *Phoma* sp., *Helminthosporium avenae*, together with unidentified fungi, bacteria, and yeasts.

TABLE 2.

The Organisms Isolated from, and the Germination in Agar of Samples 18–20 from the 1949–50 Harvest.

Sample.	Source.	Germination in Agar.	Number of Grains Giving—							
			Alternaria spp.	*Penicillium* spp.	*H. triticirepentis.*	*Septoria nodorum.*	*Aspergillus* spp.	*H. sativum.*	Miscellaneous.	No Organisms.
18	Castle Hill (Federation)	39	24	18	—	—	1	—	2	5
19	Gunnedah (Gabo)	42	40	—	6	—	—	—	3	3
20	Inverell (Gabo)	37	35	1	4	2	—	1	4	12
	Average	39·3	33·0	6·3	3·3	0·7	0·3	0·3	3·0	7·7
	Average (%)	78·7	66·0	12·7	6·7	1·3	0·7	0·7	6·0	15·4

50 grains plated from each sample.

could be placed in *A. tenuis* auct. as described by Mason (1928), Wiltshire (1933), Groves and Skolko (1944), which is a little more comprehensive than Neergaard's (1945) *A. tenuis* auct. sensu stricto. Some isolates, however, produced only short chains of conidia and there seems to be little provision made in the literature for these forms.